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Research Progress and Forecast Report
for
Contract F49620-84-C-0074

"Mechanisms of Cellular Membrane Effects of TCDD, Selected Perfluorinated Acids and Polyhalogenated Aromatic Hydrocarbons"

Submitted To:

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1 February 1985

Research Progress and Forecast Report
Contract F49620-84-C-0074
"Mechanisms of Cellular Membrane Effects of TCDD, Selected Perfluorinated Acids and Polyhalogenated Aromatic Hydrocarbons".
February 1, 1985

The first six months of the contract have been devoted to examining the effects of perfluorinated acids and polyhalogenated aromatic hydrocarbons on the colony-forming ability of L5178Y cells. Two sub clones of this cell line have been utilized. One sub clone is designated L5178Y TK+/- and this line was obtained from Dr. D. Clive, Burroughs Wellcome, North Carolina. The second sub clone is designated L5178Y TK+/+ and was obtained from Dr. C. Arlett, MRC Cell Mutation Unit, England. The two cell lines differ markedly in their growth characteristics. L5178Y TK+/- cells tend to associate in clumps of cells and are grown in a shaker incubator to produce better cell suspensions. L5178Y TK+/+ cells grow as a single cell suspension without agitation.

The toxic response of both cell lines after treatment with the perfluorinated acids (perfluoro-n-octanoic acid, 9-H hexadecafluoro-n-nonanoic acid, and perfluoro-n-decanoic acid) was measured. Two different cell media were used in the experiments to determine if the growth milieu of the cells had an effect on clonal or suspension toxicity. Experiments were conducted in Fischer's medium and in McCoy's 5A medium. The same lot of horse serum was used throughout the experiments to eliminate the effect of serum on toxicity.

The results for perfluoro-n-octanoic acid (PFOA) in both cell lines are presented in Table 1. For both cell lines and in both types of media, there was no apparent dissociation of suspension growth from clonal growth. These results are in agreement with results obtained by Andersen et. al (1983). The results for 9-H hexadecafluoro-n-nonanoic acid (9-HFNA) (Table 2) indicate that, for the TK+/- cells there is a reproducible dissociation of suspension growth from colony growth at 100 ug/ml. The effect is not observed at concentrations of 50 ug/ml or less. The effect is also seen at 100 ug/ml in the TK+/+ cells. However, this concentration produces more toxicity in suspension in TK+/+ cells when the responses of the TK+/+ and TK+/- cells are compared. There is some dissociation of colony growth from suspension at 50 ug/ml in the TK+/+ cells but not in the TK+/- cells.

The results for the perfluoro-n-decanoic acid (PFDA) are presented in Table 3. A dose-response relationship for toxicity and the ability to form colonies in soft agar was noted in both cell lines. This effect was apparent at 500, 400, 300, 200, and 100 ug/ml. At concentrations of 50 ug/ml or less there was no difference between suspension growth and colony growth. These results are different from those obtained by Andersen and co-workers. In that study, complete dissociation was observed at 10 ug/ml. We have observed complete dissociation only at

concentrations of 200 ug/ml and greater in TK+/+ and at concentrations of 400 ug/ml and greater in TK+/- cells.

We have also examined the effect of decanoic acid on TK+/+ cells using McCoys 5A medium in an attempt to reproduce some studies conducted by Andersen et. al. The results are presented in Table 4. There was no apparent dissociation of suspension growth from colony growth. This result is in agreement with results obtained Andersen and co-workers.

In summary, the results for the perfluorinated acids with chain length of 9 or 10 indicate that there is some dissociation of colony growth from suspension growth. Medium type does not affect the toxicity. This would imply that these perfluorinated acids are producing toxicity through a membrane interaction. However, these results are not as clear cut as those previously obtained by Andersen et. al. The dissociation appears to occur in the TK+/+ cells at concentrations approximately 20 fold higher than those previously reported. (AW)

The optimum exposure time for two polyhalogenated aromatic hydrocarbons (PHAH) has been determined. In the presence of an Aroclor induced rat liver S9, the optimal exposure time is 4 hours. Longer exposure times were attempted, however, the S9 mix appears to be toxic to L5178Y cells if the cells are exposed for periods over four hours. In the absence of S9, the optimium exposure time was determined to be 24 hours. Two PHAH were tested, $2,2^1,4,4^1,5,5^1$ hexachlorobiphenyl and $3,3^1,4,4^1,5,5^1$ hexachlorobiphenyl. These results indicate that at the maximum soluble concentration of both PHAH (50 ug/ml) there was no significant toxicity in suspension in either TK+/+ or TK+/- cells. The results of the cloning experiments on these compounds will be available in approximately two weeks.

Preliminary toxicities have been conducted on PFDA, PFOA and the two PHAH in the ARL-TG^R cells in preparation for the metabolic cooperation studies. The dose levels for treatment of the cells in the presence of rat hepatocytes have been selected and these experiments are currently in progress.

In other areas, Dr. L. Yang, has been designated as Assistant Principal Investigator due to the resignation from Microbiological Associates, of Dr. A. Thiligar. Dr. Yang has a broad range of expertise in human cell tissue culture, <u>in vitro</u> chemical carcinogenesis and mutagenesis, immunochemistry, membrane phenomenon and biochemical instrumentation. Her Curriculum Vitae is attached to this report.

In the upcoming three months, the research on the L5178Y cell lines will be completed. In the next six months, the metabolic cooperation studies will be completed.

Table 1. Toxicity of Perfluoro-n-octanoic acid in L5178Y mouse lymphoma cells

Concentration (ug/ml)	L5178Y TK+/+				L5178Y TK+/-			
	Fischer's		McCoys SA		Fischer's		McCoys SA	
	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal	Suspension	Clona
1000	ng		ng		10%	3%	12%	10%
500	ng		ng		8%	1%	12%	8%
400	ng		ng		6%	5%	15%	12%
300	ng		ng		8%	10%	13%	14%
200	11%	6%	20%	15%	11%	6%	17%	20%
100	48%	45%	64%	59%	39%	44%	45%	41%
50	82%	78%	76%	73%	87%	84%	79%	69%
10	88%	64%	93%	75%	91%	88%	81%	78%
5.0	95%	82%	89%	79%	91%	93%	88%	91%
1.0	97%	94%	93%	95%	94%	98%	93%	95%

ng = no growth

Table 2. Toxicity of 9-H hexadecafluoro-n-nonanoic acid L5178Y mouse lymphoma cells

Concentrations (ug/ml)	L5178Y TK+/+				L5178Y TK+/-			
	Fischer's		McCoys SA		Fischer's		McCoys SA	
	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal	Suspension	Clona
100	9%	0	12%	0	17%	0	21%	0
50	18%	2%	21%	3%	56%	75%	64%	71%
10	110%	85%	98%	93%	99%	102%	101%	91%
5.0	109%	103%	100%	107%	106%	105%	99%	90%
1.0	105%	143%	104%	101%	97%	107%	91%	97%
0.5	111%	106%	106%	104%	113%	103%	103%	112%

Table 3. Toxicity of Perfluoro-n-decanoic acid in L5178Y mouse lymphoma cells

Concentration (ug/ml)	L5178Y TK+/+				L5178Y TK+/-			
	Fischer's		McCoys SA		Fischer's		McCoys SA	
	Suspension	Clonal	Suspension	Clonal	Suspension	Clonal	Suspension	Clona
500	cell	lysis	cell	lysis	cell	lysis	cell	lys
400	17%	0	21%	0	13%	0	15%	0
300	13%	0	23%	0	19%	0	18%	0
200	19%	0	21%	0	15%	0	18%	0
100	45%	38%	39%	29%	27%	2%	28%	12
50	89%	111%	78%	76%	82%	78%	91%	89
10	88%	113%	89%	95%	102%	120%	94%	98
5.0	99%	115%	98%	101%	101%	108%	110%	95
1.0	90%	110%	99%	103%	107%	119%	101%	93
0.5	98%	108%	101%	110%	105%	107%	102%	97

Table 4. Toxicity of Decanoic acid in L5178Y TK+/+ cells grown in McCoys SA medium

Concentration (ug/ml)	Suspension	Clonal
1000	cell lysis	
500	no growth	
400	8%	10%
300	13%	12%
200	16%	15%
100	27%	25%
50	66%	46%